

Photos courtesy of Dr. Jean-Pierre Wery and Eli Lilly and Co.

What Is Biotechnology?

Biotechnology is an applied biological science that involves the research, manipulation, and manufacturing of biological molecules, tissues, and living organisms. With a critical and expanding role in health, agriculture, and environmental protection, biotechnology is expected to have a significant impact on our economy and our lives in the next century. Focused on three principal areas of research — protein crystal growth, mammalian cell and tissue culture, and fundamental biotechnology — the microgravity biotechnology program has benefited from using low-gravity environments to grow protein crystals, cells, and tissues in experiments completed thus far. Marshall Space Flight Center in Huntsville, Alabama, is NASA's Microgravity Center of Excellence for biotechnology and is supported by the biotechnology program office at Johnson Space Center in Houston, Texas.

Why Conduct Biotechnology Research in Microgravity?

Gravity significantly influences attempts to grow protein crystals on Earth. Research on the space shuttle

and the Russian space station, *Mir*, has indicated that protein crystals grown in microgravity can yield substantially better structural information than can be obtained from crystals grown under the full influence of Earth's gravity. Proteins consist of thousands — or in the case of viruses, millions — of atoms, which are weakly bound together, forming large molecules. On Earth, gravity-driven phenomena such as buoyancy-induced convection (flows caused by temperature-driven density differences in a fluid) and sedimentation (the separation of materials of different densities) may inhibit crystal growth. In microgravity, convection and sedimentation are significantly reduced, allowing for the production of better and larger crystals.

The absence of sedimentation means that protein crystals do not sink to the bottom of their growth container as they do on Earth. Consequently, they are not as likely to be affected by other crystals growing in the solution. Since convective flows are also greatly reduced in microgravity, crystals grow in a much more stable environment, which may be responsible for the improved structural order of space-grown crystals. Knowledge gained from studying the process of protein crystal growth in microgravity conditions will have implications for protein crystal growth experiments on Earth.

Research also shows that mammalian cell tissues — particularly those composed of normal cells — are sensitive to conditions found in ground-based facilities used to culture (grow) them. Fluid flows and sedimentation caused by gravity can separate the cells from each other, severely limiting the number of cells that will aggregate (come together). But tissue samples grown under microgravity conditions can be larger and more representative of tissues that are actually produced inside the human body. This suggests that better control of the stresses exerted on cells and tissues can play an important role in their culture. These stresses are greatly reduced in microgravity. (See back page for more information about microgravity, or μg .)

On the cover: These images of crystals of the protein raf kinase, which is important to cancer research, compare results of ground-based crystal growth (left) to crystal growth in microgravity (right). The long, thin crystals in the right photo are approximately an order of magnitude larger than the small, needle-like crystals in the left photo. The space-grown crystals, which were grown during the second United States Microgravity Laboratory (USML-2) mission in November 1995, were the largest crystals of raf kinase ever produced. Large, uniform crystals like these generally yield better structural information when analyzed through X-ray diffraction, which in turn can lead to a better understanding of how the structure of a protein is related to its function in the human body.

Biotechnology Research Areas

Protein Crystal Growth

The human body contains over 100,000 different proteins. These proteins play important roles in the everyday functions of the body, such as the transport of oxygen and chemicals in the blood, the formation of the major components of muscle and skin, and the fighting of disease. Researchers in the area of protein

crystal growth seek to determine the structures of these proteins, to understand how a protein's structure affects its function, and ultimately to design drugs that intercede in protein activities (penicillin is a well-known example of a drug that works by blocking a protein's function). Determining protein structure is the key to the design and development of effective drugs.

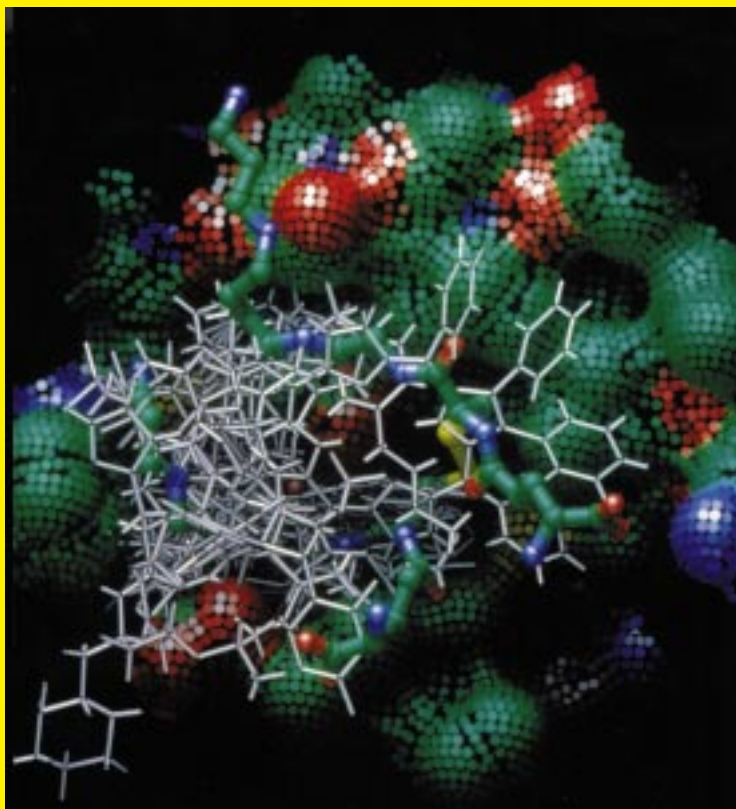
The main purpose in growing protein crystals is to advance our knowledge of

biological molecular structures.

Researchers can use the microgravity environment to help overcome a significant stumbling block in the determination of molecular structures: the difficulty of growing crystals suitable for analysis. Scientists use X-ray analysis to determine the three-dimensional structure of a protein. From high-resolution data, scientists can describe a protein's structure on a molecular scale and determine the parts of the protein that are important to its functions. Using computer analysis, scientists can create three-dimensional models of the protein and manipulate them to examine the intricacies of the protein's structure. They can then create a drug that "fits" into a protein's active site, like inserting a key into a lock, to disable the protein's function. But X-ray analysis requires large, homogeneous crystals (about the size of a grain of table salt), and unfortunately, crystals grown in the gravity environment of Earth often have internal defects that make such analysis difficult or impossible. Space shuttle missions have shown that crystals of some proteins (and other complex biological molecules, like viruses) that are grown in space, away from gravity's distortions, are larger and have fewer defects than those grown on Earth. The improved data from the analysis of space-grown crystals significantly enhance scientists' understanding of the proteins' structures, and this information can be used to support structure-based drug design.

Scientists also strive for a better understanding of the fundamental mechanisms by which proteins form crystals. A central goal of NASA's protein crystal growth program is to determine the basic science that controls how proteins interact and order themselves during the process of crystallization. The strategy for determining this is to use the knowledge gained from ground-based research and flight experiments to understand how microgravity improves the quality of protein crystals. To accomplish this goal, NASA has brought together scientists from the protein crystallography community, traditional crystal growers, and other physical scientists to form a multidisciplinary team. These collaborations are already contributing to improvements in crystal growth techniques and technology.

Defining Protein Structure



Scientists study the structure of protein crystals to determine how structure affects the function of individual proteins. To conduct this type of study, scientists must first generate crystals that are large enough and uniform enough to provide useful structural information upon analysis. Protein crystals grown in microgravity are often significantly larger and of

better quality than those grown on Earth. Once a high-quality crystal has been selected, it is examined through a process called X-ray diffraction, in which X-rays are directed into the crystal and are scattered in a regular manner by the atoms in the crystal. The scattered X-rays are recorded on photographic film or electron counters. This data is then fed into a computer, which can perform precise measurements of the intensity of the X-rays scattered by each crystal, helping scientists to map the probable positions of the atoms within each protein molecule.

Pictured above is the computer-generated model of the protein crystal trypanthione reductase, a protein that is essential to the vitality of a parasite that causes Chagas' disease, a devastating illness affecting the heart and gastrointestinal tract. Researchers can use their knowledge of the structure of such a protein to design drugs that will interact with the protein and inhibit its functions, thus preventing or curing a disease. Structure-based drug design techniques are being used in the search for treatments for diseases like Chagas', AIDS, diabetes, and cancer.

Mammalian Cell and Tissue Culture

Mammalian cell and tissue culturing is a major area of research for the biotechnology community. Tissue culturing is one of the basic tools of medical research and is key to developing future medical technologies such as *ex-vivo* (outside of the body) therapy design and tissue transplantation. To date, medical science has been unable to fully culture human tissue to the mature states of differentiation found in the body.

The study of normal and cancerous mammalian tissue growth holds enormous promise for applications in medicine. However, conventional static tissue culture methods form flat sheets of growing cells that differ in appearance and function from their three-dimensional counterparts growing in a living body. In addition, it is sometimes difficult in a static environment for growing tissue to find the fresh media (food supply) it needs to survive.

In an effort to enhance three-dimensional tissue formation, NASA scientists have developed a technology for cell and tissue culture called a rotating wall bioreactor. This instrument cultures cells in a slowly rotating horizontal cylinder, which produces lower stress levels on the growing cells than previous experimental environments designed to facilitate tissue growth

on Earth. The continuous rotation of the cylinder allows the tissue sample to be suspended in growth fluid and escape much of the influence of gravity.

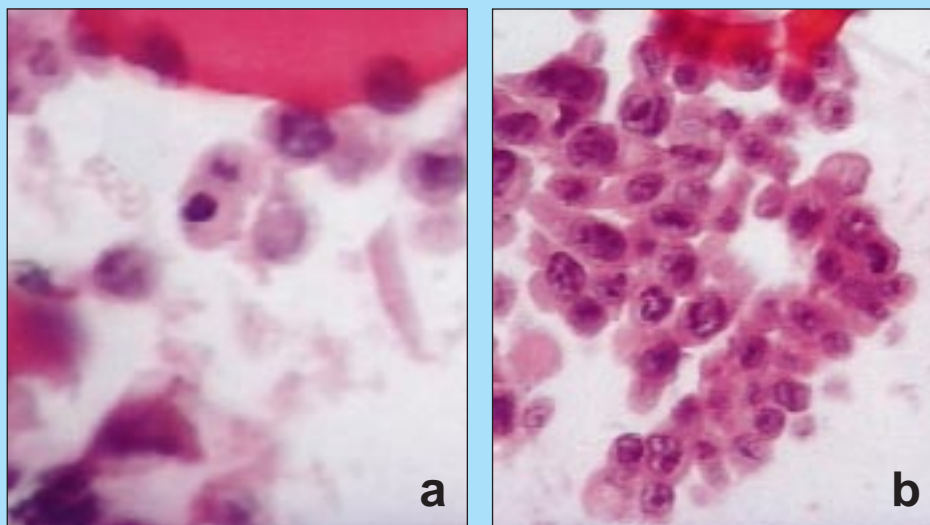
Another reason mammalian cells are sensitive to growth conditions found in standard stirred bioreactors is that fluid flow causes shear forces (forces that cause contiguous parts of a structure or solution to slide relative to each other) that discourage cell aggregation. This limits both the development of the tissue and the degree to which it possesses structures and functions similar to those found in the human body. The NASA bioreactor allows for a reduction of the damaging shear forces. Tissue cultures of the size that can be grown in this improved bioreactor allow tests of new treatments on patient cell cultures rather than on patients themselves. Scientists at Johnson Space Center are modifying the bioreactor, making it possible to automatically monitor and control levels of glucose, oxygen, pH, and carbon dioxide in the solution containing the tissue. In the future, this technology will enable quicker, more thorough testing of larger numbers of drugs and treatments. Ultimately, the bioreactor is expected to produce even better results in the microgravity environment achieved in orbit.

In cooperation with the medical community, the bioreactor design is being used to

prepare better models of human colon, prostate, breast, and ovarian tumors. Cells grown in conventional culture systems may not differentiate to form a tumor typical of cancer. In the bioreactor, however, these tumors grow into specimens that resemble the original tumor. Similar results have been observed with normal human tissues as well. Cartilage, bone marrow, heart muscle, skeletal muscle, pancreatic islet cells, liver cells, and kidney cells are examples of the normal tissues currently being grown in rotating bioreactors by investigators. In addition, laboratory models of heart and kidney diseases and viral infections (including those from the Norwalk virus, a major cause of epidemic gastroenteritis, and the human immunodeficiency virus, or HIV) are currently being developed for further study using this technology. Continued and expanded use of the bioreactor can improve our knowledge of normal and cancerous tissue development. NASA has also started to culture tissues in the bioreactor on the space shuttle and on the Russian space station, *Mir*, where even greater reduction in stresses on growing tissue samples may allow larger tissue masses to develop. Bioreactor designs for use on the International Space Station are under way.

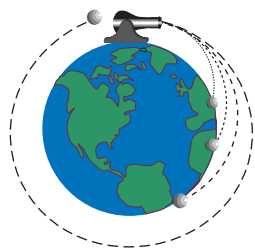
Fundamental Biotechnology

The primary purpose of research in fundamental biotechnology is to identify and understand biotechnological processes and biophysical phenomena that can be studied advantageously in a microgravity environment. Molecular and cellular aggregation, the behavior of electrically driven flows, transport processes, and capillary and surface phenomena are areas of fundamental research in biotechnology that can be applied to biological systems. Separation science and technology, particularly electrophoresis and phase partitioning, have a long history of experimentation in microgravity. Electrophoresis, which is the separation of a substance based on the electrical charge of the molecule, has been studied on a dozen space shuttle flights and has led to additional research in fluid physics.



These photos compare cultures of colon carcinoma grown on the ground (a) and in the NASA bioreactor during its first spaceflight in 1995 (b). The cells in the sample grown in microgravity have aggregated to form masses that are larger and more similar to tissue found *in vivo* than those in the ground control sample. The space-grown cells also appear to be healthier than the cells grown on Earth.

Gravity and Microgravity



In his “thought experiment,” Isaac Newton hypothesized that by placing a cannon at the top of a very tall mountain and firing a cannonball at a high enough velocity, the cannonball could be made to orbit the Earth.

Gravity is such an accepted part of our lives that we rarely think about it, even though it affects everything we do. Any time we drop or throw something and watch it fall to the ground, we see gravity in action. Although gravity is a universal force, there are times when it is not desirable to conduct scientific research under its full influence. In these cases, scientists perform their experiments in microgravity — a condition in which the effects of gravity are greatly reduced, sometimes described as “weightlessness.” This description brings to mind images of astronauts and objects floating around inside an orbiting spacecraft, seemingly free of Earth’s gravitational field, but these images are misleading. The pull of Earth’s gravity actually extends far into space. To reach a point where Earth’s gravity is reduced to one-millionth of that on Earth’s surface, one would have to be 6.37 million kilometers away from Earth (almost 17 times farther away than the Moon). Since spacecraft usually orbit only 200–450 kilometers above Earth’s surface, there must be another explanation for the microgravity environment found aboard these vehicles.

Any object in freefall experiences microgravity conditions, which occur when the object falls toward the Earth with an acceleration equal to that due to gravity alone (approximately 9.8 meters per second squared [m/s^2], or 1 g at Earth’s surface). Brief periods of microgravity can be achieved on Earth by dropping objects from tall structures. Longer periods are created through the use of airplanes, rockets, and spacecraft. The microgravity environment associated with the space shuttle is a result of the spacecraft being in orbit, which is a state of continuous freefall around the Earth. A circular orbit results when the centripetal acceleration of uniform circular motion (\mathbf{v}^2/\mathbf{r} ; \mathbf{v} = velocity of the object, \mathbf{r} = distance from the center of the object to the center of the Earth) is the same as that due to gravity alone.

Microgravity Research Facilities

A microgravity environment provides a unique laboratory in which scientists can investigate the three fundamental states of matter: solid, liquid, and gas. Microgravity conditions allow scientists to observe and explore phenomena and processes that are normally masked by the effects of Earth’s gravity.

NASA’s Microgravity Research Division (MRD) supports both ground-based and flight experiments requiring microgravity conditions of varying duration and quality. These experiments are conducted in the following facilities:

A **drop tower** is a long vertical shaft used for dropping experiment packages, enabling them to achieve microgravity through freefall. Various methods are used to minimize or compensate for air drag on the experiment packages as they fall. Lewis Research Center in

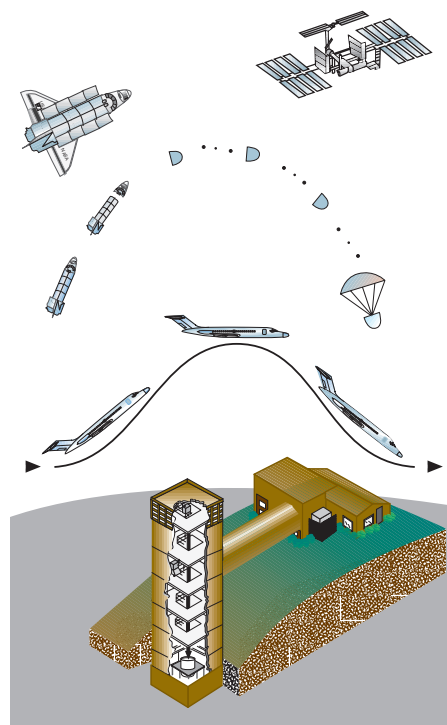
Cleveland, Ohio, has two drop facilities (one 24 meters tall and one 132 meters deep) that can accommodate experiments which need only a limited amount of time (2.2 or 5.2 seconds) in microgravity or which are test runs of experiments that will later be performed for longer periods in an aircraft, rocket, or spacecraft.

Reduced-gravity aircraft are flown in parabolic arcs to achieve longer periods of microgravity. The airplane climbs rapidly until its nose is at an approximate 45-degree angle to the horizon. Then the engines are briefly cut back, the airplane slows, and the nose is pitched down to complete the parabola. As the plane traces the parabola, microgravity conditions are created for 20–25 seconds. As many as 40 parabolic trajectories may be performed on a typical flight.

Sounding rockets produce higher-quality microgravity conditions for longer periods of time than airplanes. An experiment is placed in a rocket and launched along a parabolic trajectory. Microgravity conditions are achieved during the several minutes when the experiment is in freefall prior to re-entering Earth’s atmosphere.

A **space shuttle** is a reusable launch vehicle that can maintain a consistent orbit and provide up to 17 days of high-quality microgravity conditions. The shuttle, which can accommodate a wide range of experiment apparatus, provides a laboratory environment in which scientists can conduct long-term investigations.

A **space station** is a permanent facility that maintains a low Earth orbit for up to several decades. The facility enables scientists to conduct their experiments in microgravity over a period of several months without having to return the entire laboratory to Earth each time an experiment is completed.



**Microgravity
Research
Division**